494-Pos Board B294
Bacterial Secretion: Pore Properties of the Type III System Translocon
Beau Wager, Eric Faudry, Ina Attrée, Anne H. Delcour.
Bacteria exhibit a variety of secretion systems to export polypeptide substrates into the extracellular medium or inside eukaryotic cells. The type III secretion system (T3SS) is a needle-like proteinaceous appendage used to directly inject cytotoxins into host cells. The T3SS is comprised of about 25 different proteins spanning the bacterial cell envelope and the host cell cytoplasmic membrane. In the human pathogen, Pseudomonas aeruginosa, the PopB and PopD proteins are believed to assemble as a channel-like conduit, or translocon, in the host cell membrane for delivery of the cytotoxins in the host cytoplasm. PopB and PopD were shown to insert in the membranes of cells and artificial liposomes, where they cause permeabilization, but direct evidence of their channel function has been lacking. Here we have used the planar lipid bilayer technique to investigate the pore properties of purified PopB and PopD proteins in azolectin bilayers. PopB and an equimolar mixture of PopB and PopD readily insert into artificial bilayers. Once inserted, channel activity occurs in bursts of openings to various conductance levels. The activity is polarity dependent: very few openings are observed at voltages that are negative on the side of protein addition, and the channels inactivate rapidly as the voltage is switched from positive to negative. At positive voltages, channel activity is robust and voltage-dependent. When the PopD protein alone is added to the bilayer, little channel activity is observed, which may be due to a reluctance to insert and/or an intrinsic lower open probability. The properties of the channels formed by PopB, PopD and PopB/D mixture are compared in various conditions.

495-Pos Board B295
Endocannabinoid Inhibition of Ion Channels of Pancreatic Beta Cells
Charles Spivak, Máire E. Doyle.
The secretion of insulin from pancreatic beta cells is regulated by many factors, chiefly the plasma glucose concentration. Glucose initiates a chain of events that raises the ATP:ADP ratio within the cell. When the K(ATP) channel is inhibited by this increased ratio, the cell depolarizes, calcium action potentials ensue, and the raised cytosolic calcium concentration leads to exocytosis of insulin secretory granules. Preliminary evidence from our lab indicates that endocannabinoids are negative regulators of insulin secretion, and we previously demonstrated that the endocannabinoid 2-arachidonylglycerol (2-AG) inhibits sodium, delayed rectifier potassium, and high voltage-activated (mostly L-type) calcium channels at low micromolar concentrations in the insulinoma cell line R7T1.
In the R7T1 cell line, inside-out patches (+60 mV) displayed unitary currents of about 4.2 pA and various multiples, which were identified as K(ATP) channels by being nearly completely inhibited by 1 mM ATP. 2-AG inhibited the K (ATP) channel with an IC50 of 1 uM. This block was irreversible, even in the presence of lipid free bovine serum albumin. To test whether the block by 2-AG were mediated by the CB1 receptor, the CB1 antagonist AM-251 (10 uM) was added to the pipette solution. Though a >95% antagonism was predicted, no effect of the AM-251 was observed, suggesting that the CB1 receptor did not mediate the 2-AG blockade. This block was evident in current clamped (perforated patch) primary mouse beta cells, which showed a small (2.5 mV median) but consistent depolarization in the presence of 10 uM 2-AG. Moreover, the depolarization induced by 10 mM glucose was blunted by 10 uM 2-AG, evidence that the ion channel blockade seen in the insulinoma cells can apply to primary beta cells.

496-Pos Board B296
Dynamic Study of Ceramide Channels with a Microfluidic System
Bing Sun, **Chenren Shao**, Don L. DeVoe, Marco Colombini.
A novel microfluidic system enabling effective and robust dynamic perfusion of reagents directly to an on-chip phospholipid bilayer membrane was developed to study membrane channels. Here we report its application to the study of the dynamics of ceramide channels. The microfluidic system consists of a planar phospholipid membrane (PPM) formed across a 60-100 μm diameter aperture in a polyvinylidene chloride film formed within a thermoplastic polycarbonate substrate containing a microfabricated fluidic network. The volume of the network before the aperture is 2.3μL and the chamber beneath the aperture is 20nL. Constant pumping rates up to 5μL/min can be used without disrupting the fragile membranes and so the solution bathing the membrane can be completely replaced in less than 1 minute. Transmembrane current is monitored continuously under voltage-clamp conditions. For ceramide channel experiments, the membrane-forming solution was 5 mg diphytanoyl PC, 5 mg asolectin, 0.5 mg cholesterol, 0.13 mg C16-ceramide in 1 ml of hexanol/hexadecane (v/v 10:1). The sphingolipid, ceramide, self-assembles into large channels in phospholipid membranes and is known to play an important role in apoptosis. In our studies, channel dynamics were observed by inducing disassembly and reassembly of ceramide channels formed spontaneously in the PPMs. Perfusion with La3+ resulted in rapid disassembly. Channel reassembly occurred after washing away the La3+ using EDTA. Multiple cycles of disassembly and reassembly could be performed on the same membrane. Similar studies were performed by perfusing in Bcl-xL, a potent apoptosis inhibitor. Bcl-xL disassembles the channels rapidly but the reassembly is slow. The results are consistent with ceramide channels existing in equilibrium with ceramide monomers or aggregates in the membrane and this equilibrium can be shifted by interaction with chemical agents. Supported by grants from NIH (R21EB009485) and NSF (MCB-0641208).

497-Pos Board B297
Voltage-Gated Sodium Channel Nav1.7 Maintains the Membrane Potential and Regulates Chemokine-Induced Migration of a Subpopulation of Monocyte-Derived Dendritic Cells
Peter Hajdu, Katalin Kis-Toth, Ferenc Papp, Attila Szanto, Edit Posta, Ildiko Bacskai, Peter Gogolak, Orsolya Szilagyi, Eva Rajnavolgyi, Gyorgy Panyi.
Expression of CD1a protein defines two human dendritic cell (DC) subsets with distinct functions. We aimed to study the expression of the Nav1.7 sodium channel and the functional consequences of its activity in CD1a- and CD1a+ DC. Single-cell electrophysiology (patch-clamp) and Q-PCR experiments performed on immature sorted CD1a- and CD1a+ DC populations showed that the frequency of cells expressing Na+ current, current density and the relative expression of the SCN9A gene encoding Nav1.7 were significantly higher in CD1a+ cells than in their CD1a- counterparts. Down-regulation of Nav 1.7 expression accompanying DC maturation is abolished by increasing cytosolic Ca2+ concentration using ionomycin and thapsigargin or inhibiting the NF-κB-pathway. The activity of Nav1.7 results in a depolarized resting potential (−8.7 +/- 1.5 mV) in CD1a+ IDCs as compared to CD1a- cells lacking Nav1.7 (−47 +/- 6.2 mV) or mature DCs used as ¯controls¯ with reduced Nav1.7 expression. Silencing of the SCN9A gene shifted the membrane potential to a hyperpolarizing direction in CD1a+ immature DC resulting in decreased cell migration, similarly to the pharmacological inhibition of Nav1.7 by TTX. The control of IDC function by a voltage-gated sodium channel emerges as a new regulatory mechanism modulating the migration and other responses of these DC subsets.

498-Pos Board B298
Ion Channel Currents in the Gigaseal are Visible and have Altered Kinetics: A Finite Element Model
Chilman Bae, Vladislav S. Markin, Frederick Sachs.
The ability to form gigaseals is essential for patch clamp, but the physics of how membranes form gigaseals cannot analyzed spectroscopically because the seal region is too thin. However, ion channels located in the seal can produce measureable currents that contribute to patch recordings and potentially their kinetics can elucidate properties of the seal. We modeled the seal using a finite element simulation of the 2D cable equation with the membrane (resistance and capacitance) as one side and nonconductive/noncapacitative glass as the other, and a resistive and diffusive aqueous solution in between. The channel current was calculated as $I=g_0*C\ (x,y)/C_0*(V(x,y)-V_r)$ where g_0 is the free channel conductance, C_0 is the solution concentration of the source permeant ions that yields g_0, C(x,y), and V(x,y) are respectively the concentration of source permeant ions and the potential at position (x,y),and V_r is the reversal potential calculated from the Nernst potential. Simulations showed that to obtain 10 GΩ seals, conductivity of the seal space had to be <1/10 that of 100mMKCl, probably via increased viscosity. Steady state single channel currents decrease remarkably slowly down the seal so they contribute to observed patch clamp recordings. Rise times of single channel seal currents are slower the free channels due to capacitance and accumulation depletion of permeant ions. Because of the variation of voltage with channel location, voltage-dependent channels in the seal will exhibit different gating kinetics from free channels with shifts in the midpoint and reduced steepness of the gating curve. Changes in seal anatomy for any reason such as drug treatment will alter observed channel kinetics.

499-Pos Board B299
Functional and Molecular Characterization of Katp Channels in Fibroblasts from Normal and Infarcted Hearts
Najate Benamer, Carolina Vasquez, Karen L. Louie, William A. Coetzee, Gregory E. Morley.
Fibroblasts represent a major cell type in the heart and are essential for myocardial function. K_{ATP} channels activity has been shown to increase with in vitro differentiation of fibroblasts into myofibroblasts. The hypothesis of this study is